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Prevalence of clinical and subclinical caprine mastitis of northern region in Bangladesh

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Abstract

Mastitis is a multifactorial and infectious disease of the udder of dairy animals. Infected animals suffer from pain and fever and causing damage in udder and changes in milk. The aim of this work was to determine the prevalence of clinical and sub-clinical caprine mastitis associated with bacterial pathogens during the period from January to June, 2017. A total of 120 lactating goats were randomly selected from different villages of Dinajpur district and 240 milk samples from each udder half were aseptically collected. The udders were examined and screened for the evidence of clinical and subclinical mastitis by clinical examination of udders and examining milk samples. Subclinical mastitis was detected by California Mastitis Test (CMT). Milk samples of all clinical and subclinical mastitis goats were cultured in/on bacteriological media for isolation and characterization of responsible bacterial pathogens. The overall prevalence of clinical and subclinical mastitis in goats was found to be 11.67% and 38.75%, respectively. Bacteriological examination of mastitic milk samples of caprine revealed Staphylococcus *spp.* (32.5%) as the major pathogen followed by *Escherichia coli* (22.5%), *Pseudomonas spp.* (12.5%), *Klebsiella spp.* (12.5%) and *Bacillus spp.* (5%). Results of this study indicated that about 50% of caprine udder were infected with mastitis (38.75%), which may render lowering milk production and mortality of goats and kids. It needs to screen milk sample of lactating goats by CMT, identify mastitic goats with infected bacteria, evaluate antibiotic sensitivity and design control strategy accordingly.

Key words: Goat, CMT, mastitis, prevalence, bacteria

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Introduction

Mastitis is an inflammatory condition of udder, characterized by changes in the physical properties of the udder and physical and chemical changes in milk (Nazifi *et al*., 2011). The subclinical and clinical or acute and chronic mastitis were mostly prevalent (Raikwar and Shukla, 2015). In clinical mastitis redness, swelling, heat, and pain were present, while the sub-clinical mastitis rarely manifest any evidence of inflammation except reducing milk yield (Sarker and Samad, 2011). Subclinical mastitis is the most common in goats and is mainly caused by contagious bacteria (Persson *et al.,* 2011). Subclinical mastitis in animals is important because it constituted a reservoir of microorganisms and disseminate infection to other animals within the herd (Shearer *et al.*, 2003).

The dairy goat industry is rapidly gaining importance throughout the globe in recent years (Kumar *et al.*,

2016) including Bangladesh. Goat milk's has got tremendous nutritional values in human and given uncountable impetus to the poor farmers (Silanikove *et al.,* 2010). The obvious benefits of drinking goat milk is to aid in weight loss, reduce inflammation, optimize digestion, cheaper source of nutrients, strengthen bones and heart health, and goats milk stand second after cow milk in both temperate and tropical agriculture (Farnworth, 2002). According to FAOSTAT (2016), Bangladesh remained in the 2nd position for producing goat milk globally as it produced 17, 65,973 tons/ year. However, clinical and subclinical mastitis may adversely affect the quantity and quality of goat milk leading to financial loses (Razi *et al.,* 2012).

The microorganisms associated with mastitis have been widely studied but they are still focused of research, as the isolated species of etiological agents change over time. Over 100 species of microorganisms are involved the inflammation of udder (Hristov *et al.,* 2016). The *Staphylococcus spp*. is the commonly reported etiological agent; different bacterial isolates obtained from mastitic milk in goats were *Streptococcus spp*., *Pasteurella spp*., *E. coli, Proteus spp*., *Salmonella spp*. and *Bacillus spp*. (Islam *et al.,* 2012). Sometimes fungi, yeasts and viruses were also involved in caprine mastitis (Schalm *et al.*, 1971). There is a strong correlation between the clinical and sub-clinical cases of mastitis with the elevated levels of milk leukocytes. In context to milk, these leukocytes are named as somatic cells (Najeeb *et al.*, 2013). The indirect tests commonly used to detect milk leukocytes included California mastitis test (CMT), white side count (WST), surf filed mastitis (SFMT) as an indicator of mastitis both in cows and does (Islam *et al.,* 2012). In this study CMT was adapted to detect level of milk leukocytes in lactating goat milk. There are works onto the detection of bovine mastitis in Bangladesh but the information of caprine mastitis in the northern district of Bangladesh is scanty. This study was designed to identify the prevalence of caprine mastitis at Dinajpur district, Bangladesh with its causative bacterial agents.

Materials and Methods

***Study area and period*:** The present research was carried out on Black Bengal goats at different locations of Dinajpur city and adjacent villages of Hajee Mohammad Danesh Science and Technology University (HSTU) Campus. The laboratory works were performed at the Department of Medicine, Surgery and Obstetrics and Department of Microbiology, HSTU, Dinajpur during the period of January to June 2017.

***Survey design and sampling*:** A cross sectional observational study was conducted. A total of 120 milk samples from 60 lactating does of Dinajpur city and 120 milk samples from 60 lactating does of adjacent villages of HSTU campus were randomly selected for investigation.

***Clinical examination for the detection of mastitis*:** A thorough clinical examination of the udder and its secretions were done for the detection of clinical mastitis. Clinical examination of the lactating udders were carried out, that consisting of visual inspection and palpation of udder and teats for symmetry and size, indurations and fibrosis. Teats ends were observed for alterations such as wounds, scars, markers, vesicles, warts, patent orifices and ease of milking. Inspection of udder included visual examination posteriorly to ascertain size and disproportional symmetry. All individual quarters were observed for the abnormal consistency like firmness, presence of super-numerary or multiple teats, fibrosis, oedema, warmth and other physical defects. Clinically farms, swollen and painful udders were indicative of clinical mastitis and were noted.

***Collection of sample*:** Milk sample were collected by the owners, who were instructed to soak the teat with 70% ethanol and drying off by tissue paper, one to two drops of milks was discarded and then 10 ml of milk were taken from each quarter into labeled L or R (L for left and R for right udder halves) sterilized test tubes with rubber cap. The sample was taken first from the

teats nearest the operator before proceeding to those on the off side.

***Physical examination of milk*:** Immediately after collection, milk was subjected to physical examination with naked eyes to detect any abnormalities in color, odor, consistency, presence of blood and clot, flakes and any other visible abnormalities.

***California Mastitis Test (CMT)*:** A shallow half black paddle having four cups supplied with kit (ImmuCell, Portland, Oregon, USA) was used and was rinsed after each use. About 2 ml of milk was drawn from bottle into the cup and an estimated equal volume of CMT reagent was squirted from a polyethylene wash bottle. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane for few seconds. The reaction developed almost immediately with milk containing a high concentration of somatic cells. The peak of reaction was obtained within 10 seconds and scored. The CMT reaction/ results were read immediately as per manufacturer's recommendation and were scored for each half gland (teat) depending on the amount and thickness of gel formed as described by Shearer *et al.* (2003) In this study both the CMT score of o and Trace were considered as negative or normal while CMT scores of 1+ (weak positive), 2+ (distinct positive), 3+ (strong positive) were taken as indicators of subclinical mastitis. Somatic cell count exceeding 2.5 million/ml indicated acute and severe form of infection.

***Bacteriological examination of milk sample*:** Forty milk samples positive for mastitis were randomly selected for bacteriological examination. Each milk sample (100µl) was inoculated separately in Nutrient broth to promote bacterial growth. Following overnight incubating the broth at 37^0C, a loopful bacterial culture was streaked over nutrient agar (NA) using a sterile bacteriological loop (Cheesbrough, 1985). Repeated subculture onto the agar plate was carried out until the pure culture with homogenous colonies was obtained. Similarly, bacterial growth in nutrient broth was sub cultured on MacConkey agar, Eosin Methylene Blue agar, Staphylococcus agar no. 110, Cetrimide agar for isolation of specific bacteria. These media were incubated overnight at 37^0C and colony morphology was examined. Bacteria from the isolated colonies were stained with Gram's staining method (Merchant and Packer, 1967) to study the morphological and staining properties of the bacteria. Each isolated culture was subjected to various biochemical tests to denote specific bacterial genus or species. Based on the cultural, morphological and biochemical properties (Cheesbrough, 1985) the bacterial genus or species were identified.

***Statistical analysis*:** Chi square (χ^2) test was performed to observe the significant influence of risk factors (age, parity, litter size, lactation stage, and teat lesion) onto the causes of mastitis of goats using Statistical Package for Social Science (SPSS) Version 22.0.

Results

***Clinical examination of udder and CMT of milk samples*:** Clinical examination of 28 quarters (17 goats) showed udder and teat lesions like firmness, irregular swelling, heat, pain, sensibility, or blockage of teats indicating evidence of clinical mastitis (Figure 1a). Results of clinical examination and CMT (Figure 1c) showed clinical mastitis, subclinical mastitis and amastitis in 11.67% (n=28), 38.75% (n=93) and 49.58% (n=119) goats respectively (Figure 1). The results of SCC represented as 1+, 2+ and 3+ (Table 1) and right quarter was affected more than left quarter.

***Prevalence of clinical and subclinical caprine mastitis*:** Among 120 lactating goats examined, 69 (57.5%) were infected with mastitis; 17 were infected with clinical mastitis and 52 were subclinical mastitis. On an average 11.67%, 38.75% and 49.58% goats showed clinical, subclinical and amastitis (Figure 2) respectively. The prevalence of mastitis varied depending upon the age, parity, litter size, lactation stage and teat lesions (Table 2).

Figure 1. Mastitic udder as seen during examination of lactating goat (a). The CMT test kit used (b) and the results of CMT (c) as obtained during examination of caprine milk.

Table 1. Prevalence of subclinical mastitis in Black Bengal goats by CMT test.

Name of test	Total no of sample	Quarter side	No of sample tested	Positive results		
				1+ (%)	2+ (%)	3+ (%)
CMT	240	**L**	120	25 (20.83)	18 (15)	03 (2.5)
		R	120	23 (19.17)	20 (16.67)	04 (3.33)

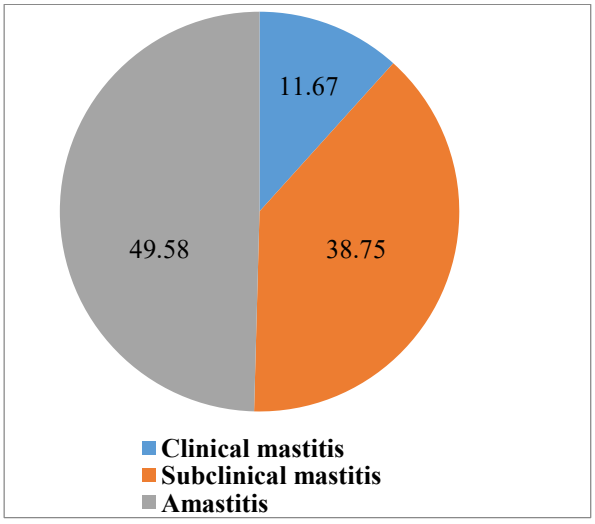

Figure 2. Overall prevalence of mastitis in lactating goat. Subclinical mastitis appeared higher than clinical mastitis in lactating goats.

The highest prevalence of clinical (23.08%) and subclinical (57.69%) mastitis was seen at the age between 4 to 5 years. A lowest prevalence (5.41%) of clinical and subclinical mastitis (35.14%) was seen at 2 to 3 years age group. The highest prevalence of clinical (24.24%) and subclinical mastitis (60.61%) cases were recorded at 5th parity and a lowest prevalence of clinical (9.09%) and subclinical (22.73%) mastitis was seen at 2nd parity. However, the first parity was not associated with clinical and subclinical mastitis. An increased prevalence of clinical and subclinical mastitis was observed with increased litter size in this study. Highest prevalence of clinical (25.81%) and subclinical mastitis (64.52%) was seen in does having 3 kids. A lowest prevalence of clinical mastitis (12.16%) in does having 2 kids and subclinical mastitis (13.33%) in does having single kid was observed. Case of clinical mastitis was not seen in does having single kids.

The highest prevalence of clinical (16.90%) and subclinical (45.07%) mastitis was detected in goats with an early lactation period, and the prevalence rate

gradually decreased as the length of lactation period was shortened. The prevalence of teat lesion was 44.74% in case of clinical mastitis and 47.37% in subclinical mastitis. Quarter having no lesions/ changes was 0% in clinical and 41.46% in subclinical mastitis.

Table 2. Prevalence of clinical and subclinical mastitis in Black Bengal goats

Sl No.	Parameters	No of goats	Clinical mastitis No (%)	Subclinical Mastitis No (%)	Amastitis No (%)	p-value	Level of Significance
1.	**Age(Years)**						
	2-3	37	2(5.41)	13(35.14)	22(59.46)		
	3-4	57	9(15.79)	24(42.11)	24(42.11)	p = 0.02	*
	4-5	26	6(23.08)	15(57.69)	05(19.23)		
	Total	120	17(14.17)	52(43.33)	51(42.5)		
2.	**Parity**						
	1st	11	00(00)	00(00)	11(100)		
	2nd	22	02(9.09)	05(22.73)	15(68.18)		
	3rd	19	02(10.53)	10(52.63)	07(36.84)	p = 0.00	**
	4th	35	05(14.29)	17(48.57)	13(37.14)		
	5th	33	08(24.24)	20(60.61)	05(15.15)		
	Total	120	17(14.17)	52(43.33)	51(42.5)		
3.	**Litter Size**						
	One	15	00(00)	02(13.33)	13(86.67)		
	Two	74	09(12.16)	30(40.54)	35(47.29)	p = 0.00	**
	Three	31	08(25.81)	20(64.52)	03(9.68)		
	Total	120	17(14.17)	52(43.33)	51(42.5)		
4.	**Lactation Stage**						
	Early	71	12(16.90)	32(45.07)	27(38.08)	p = 0.66	NS
	Mid	27	03(11.11)	12(44.44)	12(44.44)		
	Late	22	02(9.09)	08(36.36)	12(54.55)		
	Total	120	17(14.17)	52(43.33)	51(42.5)		
5.	**Teat Lesions**						
	Present	38	17(44.74)	18(47.39)	03(7.89)	p = 0.00	**
	Absent	82	00(00)	34(41.46)	48(58.54)		
	Total	120	17(14.17)	52(43.33)	51(42.5)		

* 5% level of significant ($p < 0.05$); **1% level of significant ($p < 0.01$)

***Udder halve wise prevalence of clinical and subclinical mastitis*:** Acute mastitis was detected as 11.67% (28/240) of the 28 halves (L-13, R-15) of 17 goats by clinical examination of udder and milk. Among the 17 goats, 11 had bilateral and 6 had unilateral mastitis. Subclinical mastitis was detected as 38.75% (93/240) of the 93 (L-46, R-47) halves of 52 goats by CMT. Among the 52 sub-clinically infected goats, 41 had bilateral and 11 had unilateral mastitis. Different grades of subclinical mastitis of these 93

udder halves were recorded (Table 3). Side wise prevalence of clinical mastitis recorded were 10.83% and 38.33% in the left quarter respectively. Similarly, side wise prevalence of sub-clinical mastitis recorded were 12.5% and 39.17% in the right quarter, respectively.

Table 3. Prevalence of clinical and subclinical mastitis in different quarters of udder

Types of mastitis	Quarter side	No of sample/ quarter	Positive quarter	Prevalence (%)
Clinical mastitis	**L**	120	13	10.83%
	R	120	15	12.5%
Total		240	28	11.66%
Subclinical mastitis	**L**	120	46	38.33%
	R	120	47	39.17%
Total		240	93	38.75%

***Isolation and identification of bacteria from milk samples*:** Milk samples from a total of 40 randomly selected clinical and subclinical cases of mastitis were attempted to grow in bacteriological medium (Figure 3). The bacterial isolates grown were *E. coli*, *Staphylococci*, *Pseudomonas*, *Kleibsiella*, and *Bacillus* species (Figure 4 and Table 4).

Figure 3. Isolation of bacteria in selective media like Staphylococcus media no.110 (a), MacConkey agar (b) and EMB agar (c).

Among 40 suspected milk samples tested, 34 grew single type of bacterial colony and 6 grew mixed types. The highest prevalence of *Staphylococcus spp* (n=13) was seen followed by *E. coli* (n=09), *Pseudomonas spp* (n=05), *Klebsiella spp* (n=05) and *Bacillus spp* (n=02) in terms of growing single colonies. The prevalence of *Staphylococcus spp + E. Coli, Klebsiella spp + E. Coli, Staphylococcus spp + Pseudomonas spp* were seen in 04, 01 and 01 cases respectively (Table 4).

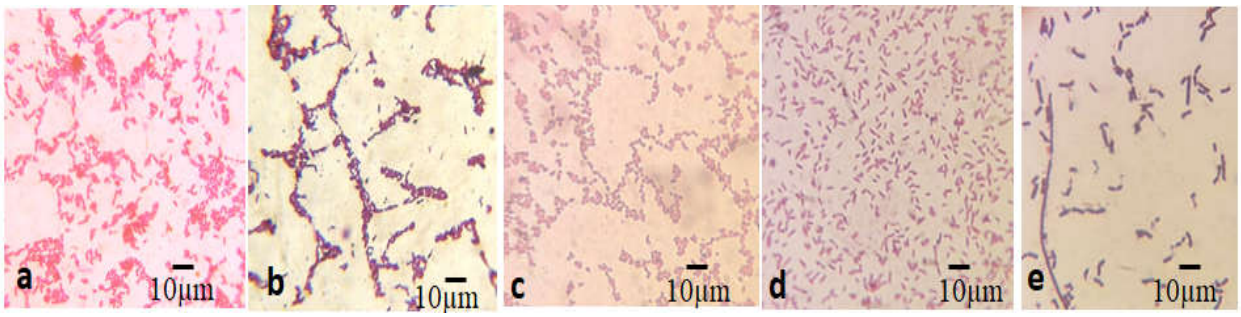

Figure 4. Bacteria isolated from the milk samples were E. coli (a), staphylococci (b), Klebsiella (c), Pseudomonas (d) and Bacillus (e) species.

Table 4. Prevalence of bacterial isolates from milk of mastitic goat

Bacterial isolates	**No. of positive cases**	**Prevalence (%)**
Staphylococcus spp.	13	32.5
Escherichia coli	09	22.5
Pseudomonas spp.	05	12.5
Klebsiella spp.	05	12.5
Bacillus spp.	02	5
Staphylococcus spp + E. coli	04	10
Klebsiella spp + E. coli	01	2.5
Staphylococcus spp + Pseudomonas spp	01	2.5

Discussion

The study showed the overall prevalence of clinical and subclinical mastitis in 11.67% and 38.75% cases respectively. Mugabe *et al.* (2017) reported a higher prevalence of sub-clinical mastitis (13.5%) than clinical mastitis (4.29%). A 10% prevalence of clinical mastitis was reported in dairy goats in Nigeria (Ameh *et al.,* 1993). Previous studies reported the prevalence of subclinical mastitis in goats were 18.29% (Rizwan *et al.,* 2016), 18.64% (Razi *et al*., 2012), 24.6% (Roukbi *et al.,* 2015), 29.92% (Al-Ramahi and Al-Nassrawi, 2007) and 36% (Islam *et al.,* 2012). The prevalence of clinical mastitis in Pakistan (3.4%, Rizwan *et al.,* 2016), Ethiopia (2.4%, Assefa *et al.,* 2006) and in Mymensingh, Bangladesh (06%, Islam *et al.,* 2012) were reported earlier but the prevalence appeared much lower than the present study. These observations revealed that the prevalence of caprine mastitis is not similar in various geographical regions. The difference in the prevalence of caprine mastitis could be due to the difference in rearing system, milking technique, breed consideration, environmental temperature, management of caprine mastitis etc. In this study the higher rate of caprine mastitis as seen may be due to fact that the infected goats seldom witness the therapeutic intervention and ultimately turn to sub clinical infection.

In this study, animal wise and quarter wise subclinical mastitis was detected in 43.33% and 38.75% cases respectively. Sharma *et al.* (2005) reported the prevalence of subclinical mastitis at 56% cases on

animal basis and 35% on quarter basis by examining 100 udder halves of 50 healthy goats in Bikaner, India. The prevalence of subclinical mastitis based on CMT in doe and udder half level was 38.92% and 28.9% respectively (Swai *et al.*, 2008).

Side wise prevalence of clinical mastitis was detected as 10.83% in left quarter and 12.5% in right quarter. Whereas, in subclinical mastitis, 38.33% in left quarter and 39.17% in right quarter was detected. Insignificant difference of affection of left half (48.2%) and right halves (51.8%) of goat udder was reported by screening 274 milk samples from 137 clinically healthy local breed does by Al-Ramahi and Al-Nassrawi (2007). However, Swai *et al.* (2008) reported a higher prevalence of mastitis in right halves (32.2%) than the corresponding left halves (25.5%). It may be due to fact that the goats which were fed to its full capacity, the rumen gets engorged and the animal tends to lie on it's right side, resulted in direct contact of right sided teats with soil and become infected (Shittu *et al.*, 2008).

In this observation, among 17 clinically affected mastitic goat, 11 had bilateral and 6 had unilateral mastitis and among 52 sub-clinically infected goats, 41 had bilateral and 11 had unilateral mastitis. Similar observation was also observed by Kumar *et al.* (2016). They examined 397 quarter foremilk samples from 200 apparently healthy lactating beetal crossbred goats and reported that 32 udder halves were unilaterally affected and 152 udder halves were bilateral affected. As opposed to this, Sarker and Samad (2011) examined 54 infected Black Bengal lactating goats and reported that 49 goats had unilateral and 5 goats had bilateral clinical mastitis. In view of several variabilities, i.e., the disposition of the quarters, milking, various managemental practices, this observation cannot be generalized, it may vary from animal to animal (Priya *et al.*, 2016) and farm to farm.

In this study, bacterial pathogen in mastitic goats were isolated by conventional methods including colony morphology, Gram's staining and different biochemical tests (Ibrahim *et al.*, 2009; Assefa *et al.*, 2006). Many infectious agents were incriminated as causes of mastitis but *Staphylococcus spp.* (32.5%), *Escherichia coli* (22.5%), *Pseudomonas spp.* (12.5%) *Klebsiella spp*. (7.5%) and *Bacillus spp.* (5%) were commonly isolated from the mastitic milk (Najeeb *et al.,* 2013; Mbindyo *et al.,* 2014; Mugabe *et al.,* 2017). The major mastitic pathogen present in the milk samples is *Staphylococcus spp.* (Kumar *et al.,* 2016; Rashid *et al.,* 2017). Earlier, the highest prevalence of Coagulase Negative Staphylococci (CNS) was reported by Gelasakis *et al.* (2016), Nickerson *et al.* (2015) and Silanikove *et al.* (2014). Other than single infection (85%), mixed infections (15%) were also found in this study which was supported by the observation of Sarker and Samad (2011). They reported that both the clinical and sub-clinical mastitis affected udder-halves had single (clinical 76.27% and sub-clinical 71.43%) and mixed (clinical 16.95% and sub-clinical 24.49%) bacterial infections. Ayodhya *et al.* (2013) also revealed mixed infection of udder due to *Staphylococcus spp., E. coli* and *Klebsiella spp.*

In this study there was a significant contribution of age (p=0.02), parity (p=0.00), litter size (p=0.00), teat lesion (p=0.00) on caprine mastitis (CM and SCM). Haftay *et al*. (2016) reported a significant contribution of age (p = 0.009) onto the prevalence of subclinical mastitis. A significantly (P>0.05) associated of parity class, breed, housing floors, flock size and suckling litter number is reported earlier (Mugabe *et al*., 2017). Gebrewahid *et al.* (2012) revealed insignificant association between risk factors and mastitis like Age (p = 0.779), parity (p = 0.201) and stage of lactation (p = 0.952). The difference in observation may be due to fact that Gebrewahid et al. (2012) carried out their study on caprine mastitis in mount region and other researchers carried out their research on plane land.

Highest prevalence of clinical (23.08%) and subclinical (57.69%) mastitis was seen at 4-5 years age group and lowest prevalence of clinical (5.41%) and subclinical (35.14%) mastitis was seen at 2-3 years age group. In

this study, an increasing trend in the prevalence of clinical and subclinical mastitis was observed with the advancement of age of the goats. Higher age group (3 years or above) is epidemiologically associated with increased subclinical mastitis of goat (Sharma *et al.,* 2007; Ali *et al.,* 2010). The increased prevalence of subclinical mastitis in older animal might be due to increase length of exposure to the pathogens compared to younger animal.

An increased prevalence of CM (24.24%) and SCM (60.61%) was found at 5th parity and lowest prevalence of CM (9.09%) and SCM (22.73%) at 2nd parity. The prevalence of subclinical mastitis was higher in animals that were at later stage of parity e.g., at the 6th and 5th parity (Boscos *et al.,* 1996; Razi *et al.,* 2012). It is assumed that at the old age, there are added burden and stress onto the body due to high milk production for longer period and multiple numbers of parity. As a result immune systems of such animals are badly affected with the infectious agents leading to mastitis (Ali *et al.*, 2010).

Highest prevalence of CM (25.81%) and SCM (64.52%) was found in does having 3 kids and lowest prevalence of CM (12.16%) recorded in does having 2 kids and SCM (13.33%) in does having single kid. The incidence of sub clinical mastitis is more in multiparous than primiparious goats (Bergonier *et al.,* 2003). The highest prevalence of CM (16.90%) and SCM (45.07%) was detected in animals with an early lactation period which was gradually decreased as the length of lactation period increased (Las Heras *et al.,* 1999; Leitner *et al.,* 2001). The higher incidence of mastitis was observed in small ruminant during suckling, suckling-milking periods or during first third of lactation in goats (Bergonier *et al.,* 2003). The highest distribution of mastitis during the early period of lactation may be due to the residual infections in udder which flare up during the early lactation period (Saxena *et al.,* 1993). Quarter having multiple lesions found to contribute CM (44.74%) and SCM (47.39%) in contrast to the quarter lacking lesion, where, 0% and 41.46% clinically and sub-clinically mastitis respectively was reported by Ali *et al.* (2010). They reported 57.7% prevalence of subclinical mastitis in the presence of teat lesion and 42.2% in the absence of teat lesion, a correlation between teat lesion and mastitis was established. Injury to the teats and udder facilitate access of microorganisms into the glands leading to mastitis (Gebrewahid *et al.,* 2012).

Conclusions

Clinical, biological and bacteriological examination of udder and milk revealed higher rate (>50%) of mastitis in lactating goats of northern region of Bangladesh. The prevalence of clinical and subclinical mastitis in Black Bengal goats were 11.67% and 38.75% respectively. The major pathogens isolated from mastitic milk were *Staphylococcus spp.* (32.5%) followed by *Escherichia coli* (22.5%), *Pseudomonas spp.* (12.5%), *Klebsiella spp.* (12.5%), *Bacillus spp.* (5%). Mixed infections were found as *Staphylococcus spp.+E. coli* (10%), *Klebsiella spp.+E. coli* (2.5%) and *Staphylococcus spp. + Pseudomonas spp.* (2.5%). The California mastitis test (CMT) can be routinely used to detect clinical and subclinical mastitis at early onset and prevent loses due to udder affections.

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